The relationship between β -catenin and patient survival in colorectal cancer: systematic review and meta-analysis.

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Running Title: B-catenin and survival in Colorectal Cancer.

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1. Introduction

Colorectal Cancer (CRC) is the third most common cancer (behind lung and breast cancer) and the second leading cause of cancer mortality worldwide [1-5]. Even though, the overall survival (OS) rate of CRC has increased due to earlier diagnosis and improved treatment strategies [4, 6], nearly half of all CRC patients still present with metastasis either at the time of diagnosis or as recurrent disease[6, 7]. In addition, the 5-year survival rate ranges from about 90% for stage I to 10% for metastatic patients [8].

During metastasis, tumour cells can spread from the primary site to a distant organ through the invasion-metastatic cascade. The epithelial–mesenchymal transition (EMT), is the process where the epithelial cell undergoes changes in its architecture and behaviour to acquire mesenchymal properties[9]. Cancer epithelial cells achieve this by undergoing biochemical changes in order to break cell-cell junctions, as well as altering the cytoskeleton and losing polarity [10]. Consequently, these cells exhibit mesenchymal characteristics such as increased evading apoptosis, increase motility and invasion [4].

A complex network of molecular signalling pathways and regulators are involved in the EMT phenomenon[11, 12]. β-catenin plays a significant role in epithelial integrity and has a dual role in tumour progression depending on its cellular localization. Membrane β-catenin interacts with the intracellular domain of E-cadherin and forms a complex which maintains cell-cell adhesion[13]. Thus, tumour cell movement and growth are restricted. Loss of E-cadherin is associated with increased cell motility due to the loss of cell adhesion, releasing β-catenin into the cytoplasm, where β--catenin accumulates and is translocated into the cell nucleus, resulting in activation of the downstream target genes causing abnormal proliferation, migration, invasion and metastasis[3, 14] (Figure 1). Moreover, both cytoplasmic and nuclear β-catenin is a main effector of canonical Wnt signalling pathway[15]. In the absence of Wnt

signals, cytoplasmic β-catenin is phosphorylated by a protein complex including axin, adenomatous polyposis coli (APC), casein kinase 1 (CK1) and glycogen synthase kinase 3 (GSK3β). Phosphorylated β-catenin is consequently ubiquitylated by ligase and degraded by the proteasome[16]. In contrast, Wnt ligands bind to Frizzled (Fzd) receptors. The complex is inactivated, preventing phosphorylation, stabilising β-catenin allowing accumulation and nuclear translocation of β-catenin. In the nucleus β-catenin binds to T-Cell Factor/Lymphoid Enhancer Factor (TCF/LEF) and activates downstream target genes such as CyclinD1, c-Myc and CD44 causing uncontrolled proliferation and migration of tumour cells. Thus, Wnt signalling pathway activation can be determined by the level of nuclear β-catenin [1, 17, 18].

Colorectal cancer is a heterogenous disease including multiple signalling alteration. Almost 60-80% of CRC develop based on an abnormal activation of Wnt signalling pathway through its central molecule, β-catenin[19-21]. Indeed, β-catenin can exist in 3 cellular locations within the cancer epithelial cell: membrane, cytoplasm, and nucleus. Membrane β-catenin forms a complex with E-cadherin to play a role in cell to cell adhesion, but when β-catenin moves to the cytoplasm under normal conditions is degraded by destruction complex. This degradation is inhibited when the Wnt ligand binds receptor, Axin translocate to the receptor complex and inhibiting destruction complex. Subsequently, the accumulated β-catenin undergoes translocation to the nucleus, where it binds a family of transcription factors (TCF/LEF) and activate Wnt target genes [22-25].

Using immunohistochemistry analysis there are several reports in the literature suggesting that expression of β-catenin could be used as a marker associated with disease progression and poor prognosis in CRC. Recent studies have reported an association between overexpression of nuclear β-catenin and distant metastasis, lymph node metastasis and poor outcome[11, 13, 17, 26, 27]. Conversely, other research reported that reduced nuclear expression was indicative of

poorer survival in CRC[15, 28]. Controversially, it has been reported that membrane or cytoplasmic β-catenin but not nuclear was associated with poor prognosis [29, 30]. Therefore, it is necessary to perform a meta-analyse for β-catenin expression in CRC patients to establish its prognostic role. In present study, we conducted a meta-analysis using published studies to investigate β-catenin expression and patient survival in CRC.

2. Materials and Methods

2.1 Literature search

The databases of Pub Med and Web of Science were searched to identify eligible studies published prior to September 2019 using the following terms and all possible combinations: ‘‘β-catenin’’, ‘‘Colorectal Neoplasms,’’ or ‘‘Colorectal Cancer’’ or ‘‘Colorectal tumour’’, “prognosis” or “survival” or “outcome”, without language limitation. The reference lists of pertinent articles were also inspected for additional related studies.

2.2 Inclusion and Exclusion Criteria

The studies were assessed for eligibility using both inclusion and exclusion criteria. Studies were considered eligible if they fulfilled the following criteria: (1) β-catenin expression evaluated in human CRC tissues using immunohistochemistry (IHC); (2) patients included had a definite pathological diagnosis of colorectal cancer; (3) examined the relationship between β-catenin expression and clinical outcome; (4) the studies investigated appropriate estimates such as hazard ratios for survival rates and their 95% confidence intervals. The following articles were excluded: (1) duplicated articles; (2) articles published in a non-English language; (3) non-human studies; (3) In vitro studies; (4) articles from which the relevant data could not be extracted.

2.3 Data Extraction

Three investigators (AM, JE and JQ) reviewed each eligible study and the following elements were extracted: first author's name, publication date, country, number of participants, age and gender, follow up duration, tumour site and stage, antibody source and dilution used, scoring method, the percent of β-catenin positivity and cellular localization. Moreover, hazard ratio (HR) and confidence interval 95% CI were collected if reported in the text for survival endpoint. Kaplan–Meier curves were used to estimate HR when it was not possible to extract HR directly from the article following the method of Tierney et al[31]. Disagreement between reviewers was resolved by discussion.

2.4 Statistical Analysis

The quantitative meta-analysis was conducted using Review Manger (Rev Man) version 5.4 and the impact of β-catenin expression in DFS, CSS and OS was measured by HRs and their 95% CIs as the pooled effective value. The definition of each outcome was as follows: DFS, the time from the date of surgery to date of record cancer progression (metastasis, recurrence or death of any cause), CCS, measured as the time from operation intervention to death from CRC, and OS, was calculated as the time from diagnosis until the date of death due to any cause. The most appropriate method was to obtain these values directly from articles. Otherwise, survival curves were used to extract these values using the previous method[31]. The I square test was used to evaluate heterogenicity among the eligible studies. Heterogenicity was considered significant at $I^2 > 50\%$ which required a random effect model. A P value < 0.05 was considered statistically significant relationship.

3. Results

3.1 Study Selection and Characteristic

A total of 948 papers were found after the initial search on Pub Med and Web of Science databases. Following removal of 270 duplicates, 480 articles were also excluded from the study based on their titles and abstracts. The remaining 198 papers were identified through scrutinizing the full text where 170 articles were excluded (45 studies lacked survival outcome, 62 studied cell lines, 14 were non-human, 48 were reviews, and one was non-English). Subsequently, the reference lists of these studies were reviewed and no additional studies for inclusion in this analysis identified. Eventually, 28 studies met the inclusion criteria and were considered eligible for meta-analysis (Figure 2).

These studies were published between 2002 and 2020, and a total of 5,475 CRC patients were enrolled and the relationship between β-catenin expression and patient survival investigated. Tables 1 to 4 illustrated all the eligible studies and summarized their characteristics. Sample sizes ranged from 66 to 903 patients and almost all of the studies (n=23) included less than 300 patients while the remaining 5 studies enrolled more than 300 patients. All publications used Immunohistochemistry to detect β-catenin expression, although the sources of primary antibodies varied. Of the 28 studies, four studies focused on membrane β-catenin whilst the others focused on nuclear β-catenin expression. The stated median age of patients ranged from 50 to 73 years in the eligible studies and ten of these studies reported a long follow up period to determine outcomes. Some of the studies defined the cut off value by combining intensity and percentage of β-catenin expression, whereas other studies used only percentage or intensity of β-catenin expression to define positive expression and the cut off value varied from 5% to 70%. In most of the studies, HR and 95 % CI were obtained from the original articles. However, the data in four studies were calculated based on the information obtained from Kaplan Meier survival curves.

3.2 Impact of β-catenin expression on overall survival (OS) of colorectal cancer

The meta-analysis was performed on 18 studies involving 4,015 patients assessing the association of nuclear β-catenin expression with OS **(**Table 3). The pooled HR for multivariable studies was significant 1.75 (95% CI: 1.21, 2.54; Z=2.94; P=0.003) while HR for univariate analysis was 1.24 (95% CI: 0.94, 1.63; P=0.13) (Figure 3) with same heterogenicity I^2 =77%. Multivariate analysis of nuclear β-catenin expression was associated with overall survival of CRC, showing it is an independent prognostic factor when compared with other variables. Therefore, multivariate was chosen in preference to univariate when both analysis were performed.

These studies used a wide variety of antibodies on Immunohistochemistry (IHC). Five studies including 1,043 patients used Dako antibody and HR was 1.54 (95% CI: 0.88, 2.67; Z=1.52; P=0.13) followed by Santa Cruz antibody (n=3 with 421 patients) which also demonstrated no significant effect with HR 1.61 (95% CI: 0.87, 3.01; Z= 1.51 P=0.13). However, Cell Signalling Technology antibody was used in two studies which involving 379 patients and resulted in significant effect of β-catenin expression on OS. Heterogeneity was detected in the Dako subgroup I^2=74% compared with moderate heterogeneity in Santa Cruz subgroup I^2 = 56% (Figure 4).

Adjuvant therapy was employed to stratify eligible studies in to two subtypes. No therapy following surgery suggested a significant relationship between poorer patient outcome and nuclear β-catenin expression with HR 1.55 (95% CI: 1.19, 2.02; Z=3.22 P=0.001) as compared to the subtype who receive adjuvant therapy after surgical intervention with HR1.38 (95% CI: 0.87, 2.19; Z=1.35 P=0.18). High heterogeneity was detected in both subtypes with I^2=60% and 80% respectively (Figure 5).

A stratification based on scoring methods was performed (Figure 6). A combined percentage and intensity subgroup (n=6 including 1,816 patients) suggested that high nuclear β-catenin

expression was associated with shorter OS HR 1.71 (95% CI: 1.08, 2.69; Z=2.31 P=0.02) with high heterogeneity I^2= 76%, while no association was observed in the intensity only subgroup (n=3 consisting of 716 patients) HR 1.07 (95% CI: 0.59, 1.91; Z= 0.21 P=0.83), and with important heterogeneity I^2=69%.

The effect of membrane β-catenin expression on OS in colorectal cancer was evaluated in four studies (n = 385 patients)[12, 32-34]. A forest plot of the individual HR estimated and resulted from meta-analysis are shown in Table 2 and Figure 7. The complete data to estimate the HR could not be retrieved from two papers and therefore were not included in the analysis. The pooled HR was 1.31 (95% CI: 1.13, 1.52; Z = 3.53 P=0.004) without heterogeneity I^2 = 1%. These studies suggested that reduce membranous β-catenin expression was statistically significant with decreased overall survival in CRC patients. However, due to the small number of studies examined, subgroup analysis was not conducted, and analysis to determine the relationship between reduced membrane β-catenin expression and overall survival was possible.

3.3 Impact of nuclear β-catenin expression on Disease Free Survival (DFS) and Cancer Specific Survival (CSS) of colorectal cancer

Six studies evaluated the relationship between β-catenin expression in colorectal cancer patients and DFS, the result demonstrated that high nuclear β-catenin predicted poorer disease-free survival (pooled HR1.66 (95% CI: 1.26, 2.17; Z=3.61 P=0.0003) (Figure 8) with a significant heterogeneity (I^2= 76% P=0.0001). Therefore, forest plot with subgroup analysis were produced to identify potential sources of heterogeneity according to adjuvant therapy were taken or not (Figure 9). Multivariate analysis is selected when both analysis done in the study. The results suggested that β-catenin overexpression in the nucleus was significantly associated to shorter disease-free survival especially in three studies (n=677) where patients did not receive

any treatment with pooled HR 1.99 (95% CI: 1.01, 3.91; Z=1.98 P=0.02) while HR for studies with adjuvant therapy was 1.20 (95% CI: 0.88, 1.63; Z=1.16 P=0.10) with significantly high and moderate heterogeneity in subgroups respectively.

CSS was examined in six studies (n=923) in relation to nuclear β-catenin expression. Intensity of colour method was used by two studies (290 patients), and the combination of intensity and percentage of positive cells was applied as scoring method in another two studies (n=366) while the remaining studies did not mention the scoring method. Subtype analysis of different scoring methods revealed no association between nuclear β-catenin expression and CSS in subgroup analysis of intensity of staining and the combination of intensity and percentage method. The studies were then stratified by treatment intervention after surgery and we found that the group who did not receive therapy showed a correlation between high nuclear β-catenin expression and shorter CSS. Notably, these results suggested that patients with high nuclear β-catenin have poorer CCS (HR 1.50 95% CI: 1.10, 2.05; Z = 2.53 P=0.01) with significantly higher heterogeneity I^2 = 71% P=0.004 (Figure 10).

4. **Discussion**

In present study, we conducted a meta-analysis to investigate β-catenin expression in CRC patients and prognosis. To reduce heterogeneity, we only included studies employing immunohistochemistry analysis. However, the scoring method, the source and dilution of primary antibody, and adjuvant therapy varied among studies, causing significant heterogeneity. The results showed that overexpression of β-catenin in the nucleus was an independent prognostic factor associated with poorer DFS, CCS and OS (Table1, 2 and 3 respectively). While alteration in membranous β-catenin was found to have a significant role in poor overall survival in CRC patient despite the small number of the studies involved in meta-analysis (Table 4). Evidence is provided and support our finding that high nuclear β-catenin

expression appeared to be associated with liver metastasis and invasion[11, 17, 35]. Nevertheless, this correlation in some studies was statistically significant in univariate analysis but not in multivariate analysis [36] [22] [37, 38], while others are significant in both analysis[22, 39]. In addition, researchers reported that no association between nuclear β-catenin overexpression and survival in CRC patients[29, 40]. These inconsistencies my exist due to limited data on the different tumour site in the same cohort and size of samples involved in the study.

Subgroup analysis of DFS, CSS, and OS revealed that nuclear overexpression of β-catenin was significantly associated with poor prognosis in non-adjuvant therapy group and there was variation between scoring methods employed. Therefore, consideration of patient stage and technical method used in scoring is an important value in the prognostic effect of β-catenin in CRC patients. Studies that combined intensity and percentage score were found to be more significant and reliable in compare to studies used intensity or percentage alone. In addition, the antibody source and dilution also affected the prognostic value reported.

Recently, reduced membranous β-catenin combined with other EMT marker (E-cadherin, Zeb 1, Snail and Fascin) was reported to allow selection of poor prognosis patient within stage II/III [4]. Additionally, a previous study shown as association between tumour spread and poor prognosis in CRC with high expression of CD44, MMP7 & β-catenin combined with low expression of KAI1/CD82 [11]. It could be postulated to have a more consistent results, multiple antibodies should be employed.

5. Conclusion

This meta-analysis revealed that overexpression of nuclear β-catenin might be an important predictor for OS, DFS, and CSS in colorectal cancer. Whereas reduce membranous β-catenin

has an effect in OS of CRC patients. However, there are limitations of present study: 1.the divergence in primary antibody and scoring method; 2. β-catenin subcellular localisation; 3. the difference between CRC patients involved in study even if they are in the same stage of disease; and 4. less precision of reconstructed HR from survival curve or table instead of directly acquired from original data. Therefore, further work is required to establish the prognostic value of β-catenin expression in patient with CRC. Also, how this expression is associated with stage of disease and the tumour microenvironment. This will require examination of β-catenin expression in large well characterised patient cohorts.

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Compliance with ethical standards

Conflict of interest: All authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. All analyses are based on previously published papers. Therefore, no ethical approval and patient consent are requiring.

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Figures legends:

Figure 1: Cartoon showing β-catenin subcellular activation of Wnt target genes.

Figure 2: Flowchart of the literature search and study selection procedure.

Figure 3: Forrest plot of HR and 95% CI for the association of β-catenin expression with OS in Colorectal patients stratified by analysis method.

Figure 4: Forrest plot of HR and 95% CI for the association of β-catenin expression with OS in colorectal cancer patients stratified by antibody.

Figure 5: Forrest plot of HR and 95% CI for the association of β-catenin expression with OS in colorectal cancer patients stratified by using adjuvant therapy.

Figure 6: Forrest plot of HR and 95% CI for the association of β-catenin expression with OS in colorectal cancer patients stratified by scoring method.

Figure 7: Forrest plot of hazard ratio for the association of Membrane β-catenin expression with OS of patients with Colorectal Cancer (CRC).

Figure 8: Forrest plot of hazard ratio for the association of β-catenin expression with DFS of patients with Colorectal Cancer (CRC) stratified by analysing method.

Figure 9: Forrest plot of hazard ratio for the association of β-catenin expression with DFS of patients with Colorectal Cancer (CRC) stratified by using adjuvant therapy.

Figure 10: Forrest plot of hazard ratio for the association of β-catenin expression with Cancer Specific Survival (CSS) of patients with Colorectal Cancer (CRC).

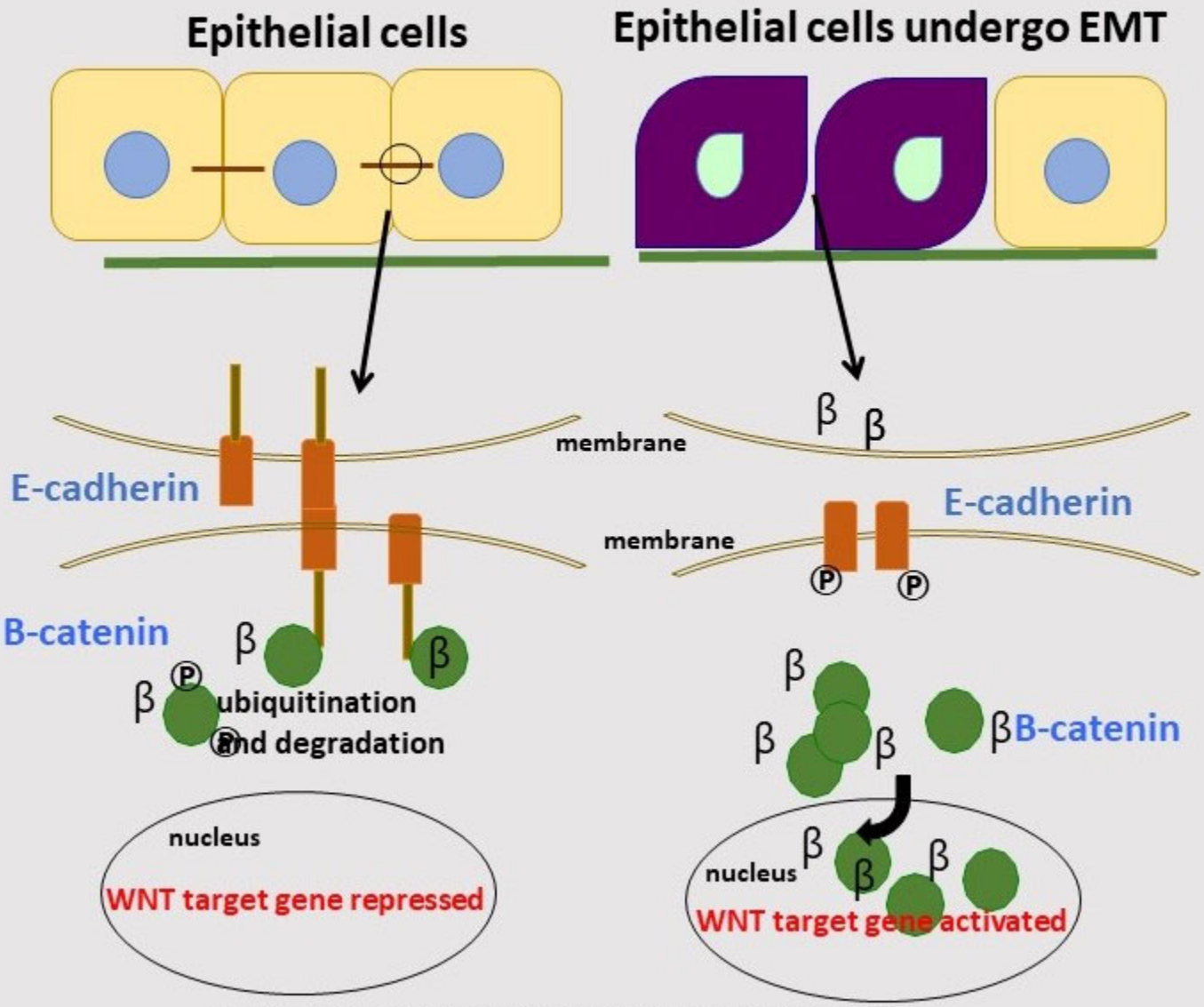


Figure 1: Role of β-catenin in subcellular activation of Wnt target genes.

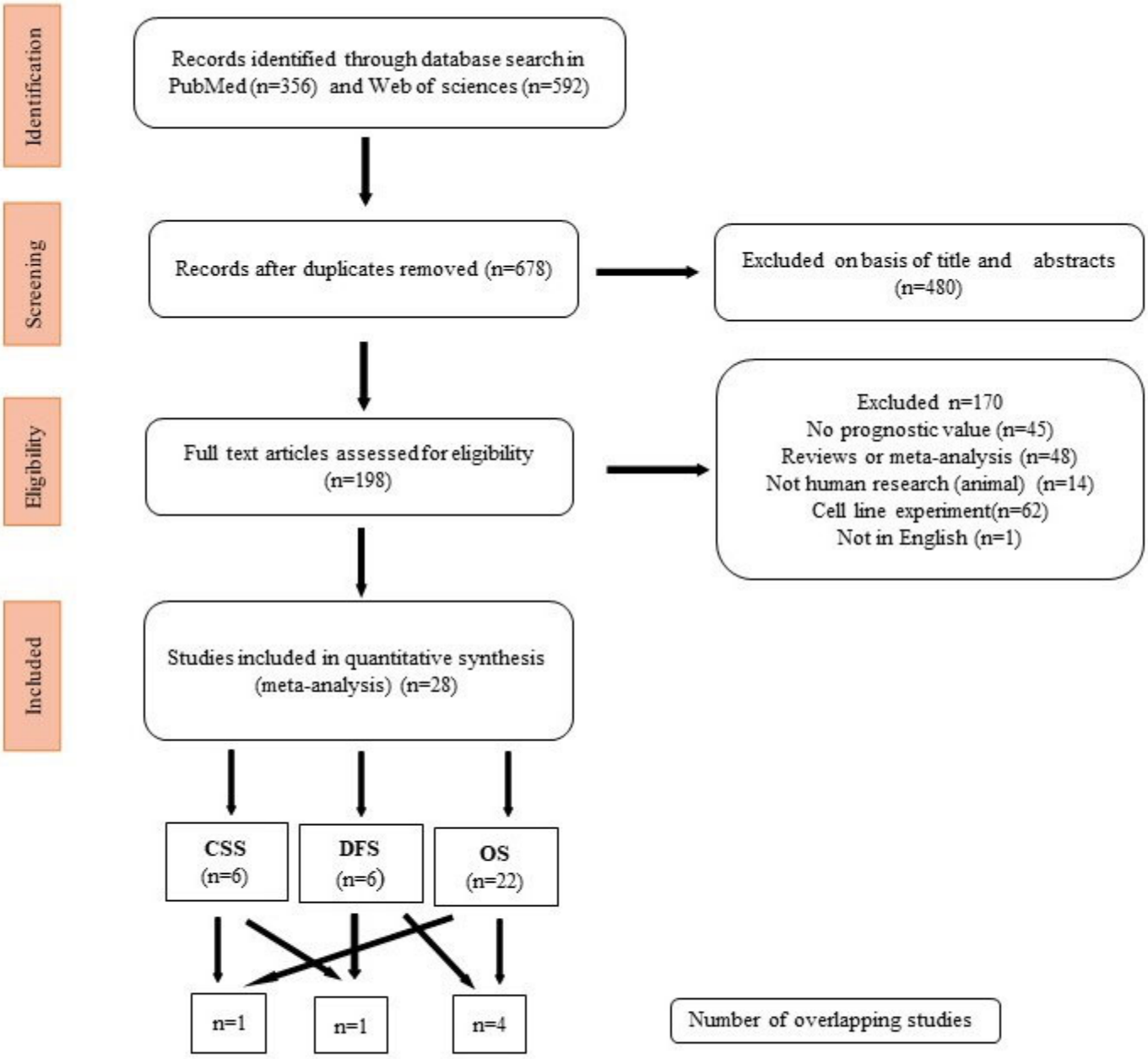


Fig. 1. Flowchart of the literature search and study selection procedure.

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI	Year
5.1.1 OS MV					
Ougolkov 2002	0.8502	0.3829	3.5%	2.34 [1.10, 4.96]	2002
Pancione 2009	1.4004	0.5097	2.7%	4.06 [1.49, 11.02]	2009
Stanczak 2011	0.9083	0.3305	3.9%	2.48 [1.30, 4.74]	2011
Jung 2013	-0.3783	0.2848	4.3%	0.69 [0.39, 1.20]	2013
Wang 2014	1.0508	0.3193	4.0%	2.86 [1.53, 5.35]	2014
Bruun 2014	-0.0249	0.162	5.3%	0.98 [0.71, 1.34]	2014
Wu 2015	0.502	0.2274	4.8%	1.65 [1.06, 2.58]	2015
Yoshida 2015	0.6419	0.3125	4.1%	1.90 [1.03, 3.51]	2015
Mojarad 2015	1.346	0.5075	2.7%	3.84 [1.42, 10.39]	2015
Lee 2016	-0.3011	0.2031	5.0%	0.74 [0.50, 1.10]	2016
Veloudis 2017	1.3507	0.5806	2.3%	3.86 [1.24, 12.05]	2017
Subtotal (95% CI)			**42.6%**	**1.75 [1.21, 2.54]**	
Heterogeneity: Tau² = 0.28; Chi² = 43.61, df = 10 (P < 0.00001); I² = 77%					
Test for overall effect: Z = 2.95 (P = 0.003)					
5.1.2 OS UV					
Ougolkov 2002	1.2267	0.3797	3.5%	3.41 [1.62, 7.18]	2002
Baldus 2004	0.4511	0.2126	4.9%	1.57 [1.04, 2.38]	2004
Bondi 2004	0.3436	0.1564	5.4%	1.41 [1.04, 1.92]	2004
Fang 2010	0.6098	0.3111	4.1%	1.84 [1.00, 3.39]	2010
Jang 2012	-0.5834	0.2543	4.6%	0.56 [0.34, 0.92]	2012
Jung 2013	-0.7072	0.2655	4.5%	0.49 [0.29, 0.83]	2013
Lee 2014	0.6419	0.3275	3.9%	1.90 [1.00, 3.61]	2014
Bruun 2014	0.1044	0.1322	5.5%	1.11 [0.86, 1.44]	2014
Mojarad 2015	0.9501	0.3944	3.4%	2.59 [1.19, 5.60]	2015
Yoshida 2015	0.7419	0.288	4.3%	2.10 [1.19, 3.69]	2015
Balzi 2015	0.0198	0.2466	4.6%	1.02 [0.63, 1.65]	2015
Lee 2016	-0.4065	0.2012	5.0%	0.67 [0.45, 0.99]	2016
Chang 2020	-0.0726	0.3641	3.7%	0.93 [0.46, 1.90]	2020
Subtotal (95% CI)			**57.4%**	**1.24 [0.94, 1.63]**	
Heterogeneity: Tau² = 0.18; Chi² = 51.52, df = 12 (P < 0.00001); I² = 77%					
Test for overall effect: Z = 1.51 (P = 0.13)					
Total (95% CI)			**100.0%**	**1.42 [1.14, 1.77]**	
Heterogeneity: Tau² = 0.21; Chi² = 97.17, df = 23 (P < 0.00001); I² = 76%					
Test for overall effect: Z = 3.17 (P = 0.002)					
Test for subgroup differences: Chi² = 2.16, df = 1 (P = 0.14), I² = 53.7%					

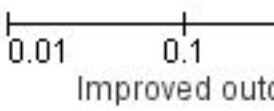


Figure 3. Forrest plot of HR and 95% CI for the association of β-catenin expression wi
patients stratified by analysis method.

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI	Year
5.3.1 Dako					
Fang 2010	0.6098	0.3111	13.5%	1.84 [1.00, 3.39]	2010
Stanczak 2011	0.9083	0.3305	12.9%	2.48 [1.30, 4.74]	2011
Jung 2013	-0.3783	0.2848	14.2%	0.69 [0.39, 1.20]	2013
Balzi 2015	0.0198	0.2466	15.4%	1.02 [0.63, 1.65]	2015
Mojarad 2015	1.346	0.5075	8.7%	3.84 [1.42, 10.39]	2015
Subtotal (95% CI)			**64.7%**	**1.54 [0.88, 2.67]**	
Heterogeneity: Tau² = 0.29; Chi² = 15.59, df = 4 (P = 0.004); I² = 74%					
Test for overall effect: Z = 1.52 (P = 0.13)					
5.3.2 Santa Cruz					
Wu 2015	0.502	0.2274	15.9%	1.65 [1.06, 2.58]	2015
Veloudis 2017	1.3507	0.5806	7.4%	3.86 [1.24, 12.05]	2017
Chang 2020	-0.0726	0.3641	12.0%	0.93 [0.46, 1.90]	2020
Subtotal (95% CI)			**35.3%**	**1.61 [0.87, 3.01]**	
Heterogeneity: Tau² = 0.17; Chi² = 4.52, df = 2 (P = 0.10); I² = 56%					
Test for overall effect: Z = 1.51 (P = 0.13)					
Total (95% CI)			**100.0%**	**1.55 [1.05, 2.28]**	
Heterogeneity: Tau² = 0.20; Chi² = 20.50, df = 7 (P = 0.005); I² = 66%					
Test for overall effect: Z = 2.20 (P = 0.03)					
Test for subgroup differences: Chi² = 0.01, df = 1 (P = 0.91), I² = 0%					

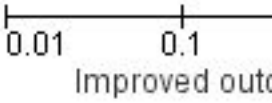


Figure 4. Forrest plot of HR and 95% CI for the association of β-catenin expression wi
cancer patients stratified by antibody.

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI	Year
5.4.1 Adjuvant Therapy					
Pancione 2009	1.4004	0.5097	3.5%	4.06 [1.49, 11.02]	2009
Fang 2010	0.6098	0.3111	5.5%	1.84 [1.00, 3.39]	2010
Stanczak 2011	0.9083	0.3305	5.3%	2.48 [1.30, 4.74]	2011
Jang 2012	-0.5834	0.2543	6.2%	0.56 [0.34, 0.92]	2012
Wang 2014	1.0508	0.3193	5.4%	2.86 [1.53, 5.35]	2014
Balzi 2015	0.0198	0.2466	6.3%	1.02 [0.63, 1.65]	2015
Lee 2016	-0.3011	0.2031	6.9%	0.74 [0.50, 1.10]	2016
Chang 2020	-0.0726	0.3641	4.9%	0.93 [0.46, 1.90]	2020
Subtotal (95% CI)			**44.0%**	**1.38 [0.87, 2.19]**	
Heterogeneity: Tau² = 0.35; Chi² = 35.32, df = 7 (P < 0.00001); I² = 80%					
Test for overall effect: Z = 1.35 (P = 0.18)					
5.4.2 No adjuvant therapy					
Ougolkov 2002	0.8502	0.3829	4.7%	2.34 [1.10, 4.96]	2002
Baldus 2004	0.4511	0.2126	6.8%	1.57 [1.04, 2.38]	2004
Bondi 2004	0.3436	0.1564	7.5%	1.41 [1.04, 1.92]	2004
Jung 2013	-0.3783	0.2848	5.8%	0.69 [0.39, 1.20]	2013
Lee 2014	0.6419	0.3275	5.3%	1.90 [1.00, 3.61]	2014
Bruun 2014	-0.0249	0.162	7.4%	0.98 [0.71, 1.34]	2014
Yoshida 2015	0.6419	0.3125	5.5%	1.90 [1.03, 3.51]	2015
Wu 2015	0.502	0.2274	6.6%	1.65 [1.06, 2.58]	2015
Mojarad 2015	1.346	0.5075	3.5%	3.84 [1.42, 10.39]	2015
Veloudis 2017	1.3507	0.5806	3.0%	3.86 [1.24, 12.05]	2017
Subtotal (95% CI)			**56.0%**	**1.55 [1.19, 2.02]**	
Heterogeneity: Tau² = 0.10; Chi² = 22.73, df = 9 (P = 0.007); I² = 60%					
Test for overall effect: Z = 3.21 (P = 0.001)					
Total (95% CI)			**100.0%**	**1.48 [1.16, 1.88]**	
Heterogeneity: Tau² = 0.18; Chi² = 60.34, df = 17 (P < 0.00001); I² = 72%					
Test for overall effect: Z = 3.15 (P = 0.002)					
Test for subgroup differences: Chi² = 0.18, df = 1 (P = 0.67), I² = 0%					

Figure 5. Forrest plot of HR and 95% CI for the association of β-catenin expressio
cancer patients stratified by using adjuvant therapy.

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI	Year
5.5.1 Intensity					
Yoshida 2015	0.6419	0.3125	10.2%	1.90 [1.03, 3.51]	2015
Lee 2016	-0.3011	0.2031	12.4%	0.74 [0.50, 1.10]	2016
Chang 2020	-0.0726	0.3641	9.2%	0.93 [0.46, 1.90]	2020
Subtotal (95% CI)			**31.7%**	**1.07 [0.59, 1.91]**	
Heterogeneity: Tau² = 0.18; Chi² = 6.42, df = 2 (P = 0.04); I² = 69%					
Test for overall effect: Z = 0.21 (P = 0.83)					
5.5.2 Intensity and Percentage					
Pancione 2009	1.4004	0.5097	6.8%	4.06 [1.49, 11.02]	2009
Jung 2013	-0.3783	0.2848	10.7%	0.69 [0.39, 1.20]	2013
Wang 2014	1.0508	0.3193	10.0%	2.86 [1.53, 5.35]	2014
Lee 2014	0.6419	0.3275	9.9%	1.90 [1.00, 3.61]	2014
Bruun 2014	-0.0249	0.162	13.1%	0.98 [0.71, 1.34]	2014
Wu 2015	0.502	0.2274	11.9%	1.65 [1.06, 2.58]	2015
Veloudis 2017	1.3507	0.5806	5.8%	3.86 [1.24, 12.05]	2017
Subtotal (95% CI)			**68.3%**	**1.71 [1.09, 2.69]**	
Heterogeneity: Tau² = 0.26; Chi² = 25.03, df = 6 (P = 0.0003); I² = 76%					
Test for overall effect: Z = 2.31 (P = 0.02)					
Total (95% CI)			**100.0%**	**1.46 [1.03, 2.08]**	
Heterogeneity: Tau² = 0.22; Chi² = 34.85, df = 9 (P < 0.0001); I² = 74%					
Test for overall effect: Z = 2.10 (P = 0.04)					
Test for subgroup differences: Chi² = 1.56, df = 1 (P = 0.21), I² = 35.9%					

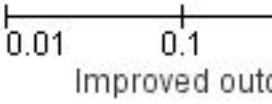


Figure 6. Forrest plot of HR and 95% CI for the association of β-catenin expression wi
cancer patients stratified by scoring method.

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI	Year
6.1.1 OS using membrane expression					
Boo 2007	0.2151	0.0979	57.6%	1.24 [1.02, 1.50]	2007
Zhang 2008	0.3286	0.1631	21.4%	1.39 [1.01, 1.91]	2008
Andras 2012	0.5653	0.225	11.4%	1.76 [1.13, 2.74]	2012
Toth 2012	0.3293	0.3048	6.2%	1.39 [0.76, 2.53]	2012
Balzi 2015	-0.2877	0.4101	3.4%	0.75 [0.34, 1.68]	2015
Subtotal (95% CI)			**100.0%**	**1.31 [1.13, 1.52]**	
Heterogeneity: Tau² = 0.00; Chi² = 4.05, df = 4 (P = 0.40); I² = 1%					
Test for overall effect: Z = 3.53 (P = 0.0004)					
Total (95% CI)			**100.0%**	**1.31 [1.13, 1.52]**	
Heterogeneity: Tau² = 0.00; Chi² = 4.05, df = 4 (P = 0.40); I² = 1%					
Test for overall effect: Z = 3.53 (P = 0.0004)					
Test for subgroup differences: Not applicable					

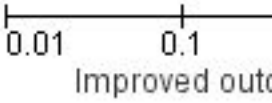


Figure 7. Forrest plot of hazard ratio for the association of Membrane β-catenin expres
with Colorectal Cancer (CRC).

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI	Year
4.1.1 DFS MV					
Ougolkov 2002	1.0438	0.3909	8.0%	2.84 [1.32, 6.11]	2002
Horst 2009	1.0716	0.4102	7.5%	2.92 [1.31, 6.52]	2009
Wang 2014	0.1451	0.047	21.6%	1.16 [1.05, 1.27]	2014
Yoshida 2015	0.7419	0.3189	10.2%	2.10 [1.12, 3.92]	2015
Subtotal (95% CI)			**47.3%**	**1.95 [1.11, 3.42]**	
Heterogeneity: Tau² = 0.24; Chi² = 13.26, df = 3 (P = 0.004); I² = 77%					
Test for overall effect: Z = 2.33 (P = 0.02)					
4.1.2 DFS UV					
Ougolkov 2002	1.4725	0.385	8.2%	4.36 [2.05, 9.27]	2002
Lee 2013	0.207	0.0965	20.0%	1.23 [1.02, 1.49]	2013
Balzi 2015	-0.0834	0.2091	14.7%	0.92 [0.61, 1.39]	2015
Yoshida 2015	0.8755	0.3301	9.8%	2.40 [1.26, 4.58]	2015
Subtotal (95% CI)			**52.7%**	**1.68 [1.00, 2.82]**	
Heterogeneity: Tau² = 0.21; Chi² = 16.39, df = 3 (P = 0.0009); I² = 82%					
Test for overall effect: Z = 1.96 (P = 0.05)					
Total (95% CI)			**100.0%**	**1.66 [1.26, 2.17]**	
Heterogeneity: Tau² = 0.09; Chi² = 30.38, df = 7 (P < 0.0001); I² = 77%					
Test for overall effect: Z = 3.66 (P = 0.0003)					
Test for subgroup differences: Chi² = 0.14, df = 1 (P = 0.70), I² = 0%					

Figure 8. Forrest plot of hazard ratio for the association of β-catenin expression wi
Colorectal Cancer (CRC) stratified by analysing method

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI	Year
2.1.2 Adjuvant therapy					
Wang 2014	0.1451	0.047	32.4%	1.16 [1.05, 1.27]	2014
Balzi 2015	-0.0834	0.2091	16.4%	0.92 [0.61, 1.39]	2015
Yoshida 2015	0.7419	0.3189	9.7%	2.10 [1.12, 3.92]	2015
Subtotal (95% CI)			**58.5%**	**1.20 [0.88, 1.63]**	
Heterogeneity: Tau² = 0.04; Chi² = 4.70, df = 2 (P = 0.10); I² = 57%					
Test for overall effect: Z = 1.16 (P = 0.25)					
2.1.3 Non adjuvant therapy					
Ougolkov 2002	1.0438	0.3909	7.1%	2.84 [1.32, 6.11]	2002
Horst 2009	1.0716	0.4102	6.6%	2.92 [1.31, 6.52]	2009
Lee 2013	0.207	0.0965	27.8%	1.23 [1.02, 1.49]	2013
Subtotal (95% CI)			**41.5%**	**1.99 [1.01, 3.91]**	
Heterogeneity: Tau² = 0.26; Chi² = 8.09, df = 2 (P = 0.02); I² = 75%					
Test for overall effect: Z = 1.98 (P = 0.05)					
Total (95% CI)			**100.0%**	**1.36 [1.08, 1.71]**	
Heterogeneity: Tau² = 0.04; Chi² = 14.92, df = 5 (P = 0.01); I² = 66%					
Test for overall effect: Z = 2.62 (P = 0.009)					
Test for subgroup differences: Chi² = 1.76, df = 1 (P = 0.19), I² = 43.1%					

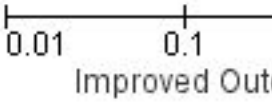


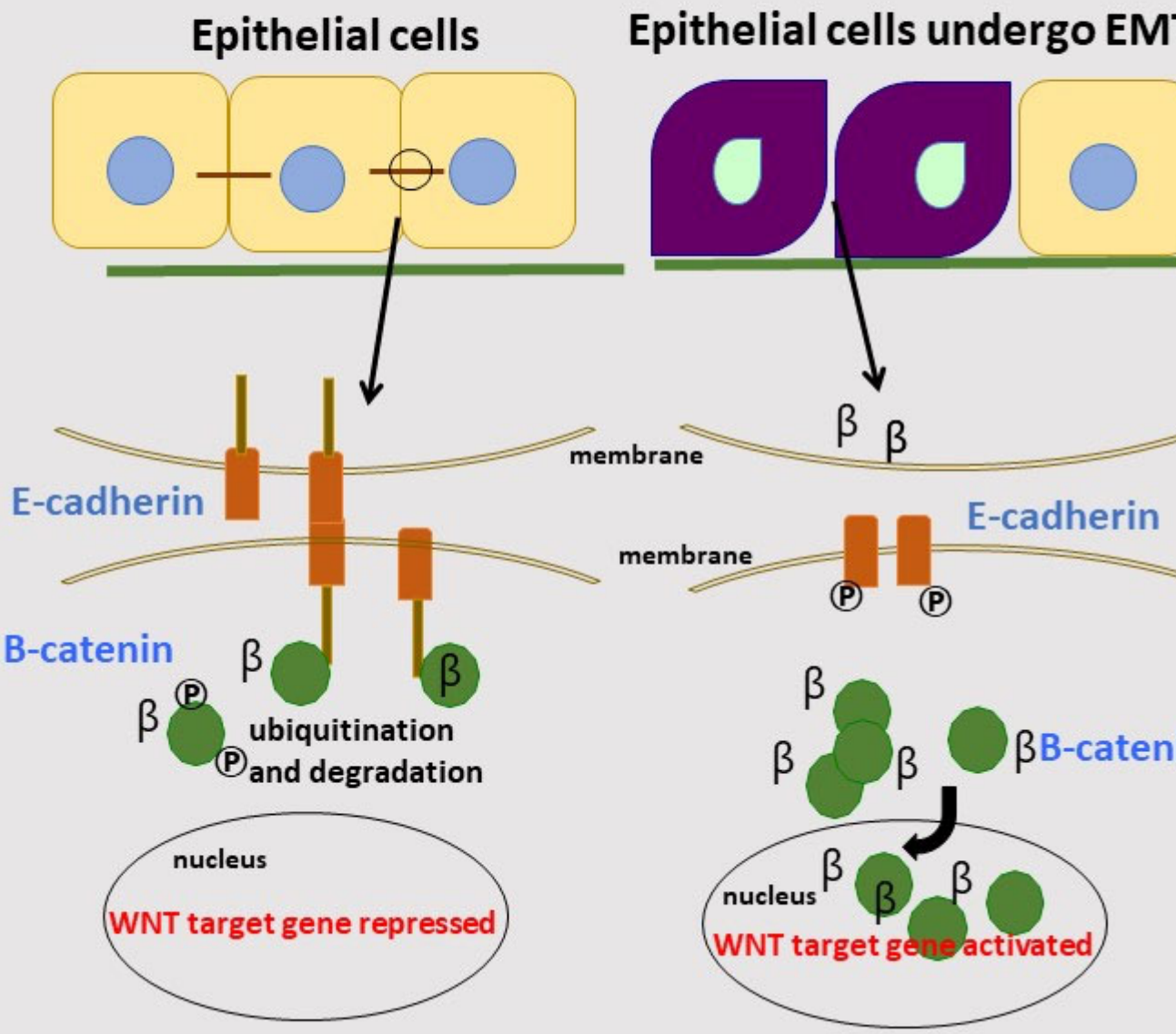
Figure 9: Forrest plot of hazard ratio for the association of β-catenin expression with I
Colorectal Cancer (CRC) stratified by using adjuvant therapy

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI	Year
Cheah 2002	0.7031	0.2361	18.4%	2.02 [1.27, 3.21]	2002
Horst 2009	2.0096	0.6516	5.0%	7.46 [2.08, 26.75]	2009
Matsuoka 2011	0.5642	0.2676	16.5%	1.76 [1.04, 2.97]	2011
Gao 2014	0.2852	0.1226	26.0%	1.33 [1.05, 1.69]	2014
Roseweir 2019	0.0862	0.0813	28.4%	1.09 [0.93, 1.28]	2019
Chang 2020	-0.3011	0.6038	5.7%	0.74 [0.23, 2.42]	2020
Total (95% CI)			**100.0%**	**1.50 [1.10, 2.05]**	

Heterogeneity: Tau² = 0.08; Chi² = 17.12, df = 5 (P = 0.004); I² = 71%

Test for overall effect: Z = 2.53 (P = 0.01)

Figure 10. Forrest plot of hazard ratio for the association of β-catenin expression w
(CSS) of patients with Colorectal Cancer (CRC).



Cartoon showing β-catenin subcellular activation of Wnt target genes..

Table 1. Studies characteristics and the impact of β -catenin expression on Disease Free Survival (DFS) in Colorectal Cancer.

Author(s)	Yoshida [26]	Balzi [20]	Wang [5]	Lee [40]	Horst [39]	Ougolkov [36]
Year	2015	2015	2014	2013	2009	2002
Country	Japan	Italy	China	Korea	Germany	Japan
Patients (N)	201	412 (321)	178	333	142	202
Age (years)	Mean 66.7	NA	Mean 64	Mean 63.6	Median 69	Mean 60
Gender (M/F)	120/81	171/150	126/52	189/144	71/71	110 / 92
Follow up (months)		Median 63.6	Median 54		Median 72	
Neo adjuvant /Adjuvant Therap	adjuvant (79)	adjuvant (159)	adjuvant			
Cancer related death n(%)	48	NA			15	
High Beta-catenin expression r	82 N	156 N	93 N b-catenin at the invasive front	38 N	N	107 N (Nainv 18)
Tumour stage	Stage II & III	Stage I -II & III	Stage III	Stage I-II-III&IV	Stage II	
Tumour site	Colon 107 Rectum 94	Colon 200 Rectum 121		Right 66 Left 236		cecum and ascending colon 53 transverse colon 21 descer colon 18 sigmoid colon 110
Scoring Method	intensity		percentage and intensity tissue section	Percentage		
Score range and location	0-1	0-1	0-1	0-1	0-1	
DOP		5% cut off value	>50%	>30%		
Antibody for IHC dilution	1;100	1;200	1;50	1;400		1;100
Antibody Source	Cell Signalling Technology	Dako	cell signalling technology	Dako	Ventana Medical Systems,	Transduction Laboratories
MV Variables	Age, Tumour location, Tumour depth, Lymph node metastasis, Tumour differentiation, Lymphatic invasion, Venous invasion, Adjuvant chemother Wnt1, Wnt3,Wnt5a, Wnt8a, Membran and Cytoplasm β--catenin expression.	NA	Gender, Age, Tumour Siz, Type of Tumour, Differentiation, Invasion depth, Lymph node metastasis.	NA	Age, Sex.	Age, Gender, Tumour stage.
HR (95% CI)	UV analysis 2.4 (1.2-3.8) MV analysis 2.1 (1.1-3.9)	UV analysis 0.92 (0.60-1.41)	MV analysis (0.669–1.998)	UV analysis	MV analysis 2.92 (1.30-6.53)	UV analys 4.36 (2.05–9.28) MV analysis 2.84 (1.32–6.10)
P value	0.008 0.02	0.69	0.002	0.032	0.009	0.0001 0.0076
HR estimation	Survival table	Survival table	Survival curve Survival table	Survival curve	Survival table	Survival table Survival curve

DOP; Definition of Positive, **NA**; Non applicable, **MV**; Multivarate , **UV**; Univariante. **N**; Nuclear, **HR**; Hazard Ratio, **95% CI** ;95% confidence Interval.

Table 2. Studies characteristics and the impact of β -catenin expression on Cancer Specific Survival (CSS) in Colorectal Cancer.

Author(s)	Chang [17]	Roseweir [4]	Gao [13]	Matsuoka [25]	Horst [39]	Cheah [30]
Year	2020	2019	2014	2011	2009	2002
Country	Korea	UK	China	Japan	Germany	Singapore
Patients (N)	190 (148)	274 (185)	181	156	142	111(78)
Age (years)	Median 62	N/A	Median 51	mean65 & median 66	Median 69	NA
Gender (M/F)	93/55	101/84	105/76	99/57	71/71	69/42
Follow up (months)	Median 146.4	Median 136.8	Median 51	Median 73 Mean 79	Median 72	NA
Neo adjuvant /Adjuvant Therapy	adjuvant (134)	adjuvant (60)	NA	NA	NA	NA
Cancer related death n	11	62	NA	NA	15	NA
High Beta-catenin expression n	90	97	30	N 43	NA	12
Tumour stage	Stage II	Stages II–III	Stage I-II-III&IV	Stage II, III & IV	Stage II	Stage I-II&III
Tumour site	Ascending 28 Transverse 5 Descending 50 Rectum 65	Colon (right side) 77 Colon (left side) 56 Rectum 60	Colon 72 Rectum 109	Proximal 51 Distal 105	NA	Left 91 Right 18
Scoring Method	Intensity	Percentage and intensity	percentage and intensity	percentages and intensity	NA	Intensity
Score range and location	Nuclear 0-3	Nuclear 0-300	Nuclear (0-3/0-100%) 0-12 immunoreactivity score	Nuclear 0-2	Nuclear 0-1	Nuclear 0-3(0-1)
DOP	NA	NA	NA	>5%	NA	NA
Antibody for IHC dilution	NA	1:50	1:500	1:400	NA	1:500
Antibody Source	Santa Cruz	Biosciences 610154	Abcam	Zymed Laboratories	Ventana Medical Systems,	Transduction Laboratories,
Multivariate variables	NA	NA	NA	NA	Age, Sex.	NA
Hazard ratio (95% CI)	UV analysis 0.74 (0.23-2.42)	UV analysis	UV analysis	UV analysis 1.758 (1.041–2.969)	MV analysis 7.46 (2.08-26.72)	UV analysis
P value	0.618	0.289	0.02	0.035	0.002	0.0029
HR estimation	Survival table	Survival curve	Survival curve / Survival table	Survival table	Survival table	Survival curve / reported in text

DOP; Definition of Positive, **NA**; Non applicable, **MV**; Multivarate , **UV**; Univariante, **N**; Nuclear, **HR**; Hazard Ratio, **95% CI**;95% confidence Interval

Table 3. Studies characteristics and the impact of β -catenin expression on Overall Survival (OS) in Colorectal Cancer.

Author(s)	Chang [17]	Veloudis [1]	Lee [27]	Yoshida [26]	Wu [11]	Mojarad [22]	Balzi [20]	Bruun [29]	Lee [14]	Wang [5]	Jung [37]	Jang [38]	Stanczak [23]	Fang [24]	Pancione [21]	Baldus [18]	Bondi [35]	Ougolkov [36]
Year	2020	2017	2016	2015	2015	2015	2015	2014	2014	2014	2013	2012	2011	2010	2009	2004	2004	2002
Country	Korea	Greece	Korea	Japan	China	Iran	Italy	Norway	Korea	China	Korea	Korea	Poland	China	Italy	Germany	Norway	Japan
Patients (N)	190 (148)	57	543 (367)	201	174	165	412 (321)	929 (903)	83	178	349	220	66	142	89 (72)	205	162	202
Age (years)	Median 62	NA	Mean 64.2	Mean 66.7	Median 62.1	Mean 49.25 for +ve b-cateinin. 56.24 for -ve	NA	Median 73	Median 60	Mean 64	Median 63	NA	Mean 71	Mean 55	Mean 70.5	Mean 64.96 / Median 65.57	Mean 71.1	Mean 60
Gender (M/F)	93/55	NA	205/162	120/81	101/73	85/80	171/150	429/474	46/37	126/52	208/141	134/84	44/22	80/62	44/28	102/103	74/88	110 / 92
Follow up (months)	Median 146.4	NA	Median 55	NA	Mean 51.78	Mean 47.2, Median 38	Median 63.6	NA	NA	Median 54	Mean 55.3	NA	NA	NA	Median 56	NA	Mean 50.4	NA
Neo adjuvant /Adjuvant Therapy	adjuvant (134)	No	Adjuvant (269)	adjuvant (79)	NA	NA	adjuvant (159)	NA	NA	adjuvant	No	adjuvant 109	adjuvant 25	adjuvant	adjuvant	NA	NA	NA
Cancer related death n	11	NA	NA	48	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	21		73	NA
High Beta-catenin expression n	90	14	221	82	129	32	156	637 (561)	NA	93	246	NA	NA	41	13	105	36	107
Tumour stage	Stage II CRC	Stage I-II-III&IV	Stage I-II-III&IV	Stage II & III	Stage I-II-III&IV	Stage I-II-III&IV	Stage I -II & III	Stage I-II-III&IV	NA	Stage III	Stage I-II-III&IV	Stage I, II, III & IV	NA	NA	NA	Stage I-II-III&IV	NA	NA
Tumour site	Ascending 28 Transverse 5 Descending 50 Rectum 65	Colon 41 Rectal 16	cecum 13ascending 55 hepatic flexure 22 transverse 16 splenic flexure 6 descending 18 sigmoid 114 rectum 123	Colon 107 Rectum 94	Rectum 89 Colon 85	Ascending 24 Transverse 26 Descending 27 Sigmoid 25 Rectum 63	Colon 200 Rectum 121	Proximal 367 Distalcolon302 Rectum234	Colon 51 Rectum 32	NA	Right 76 Left 273	Rt. Colon 51 Lt. Colon 64 Rectum 103	Colon 23 Sigmoid colon 24 Rectum 19	Colon 75 Rectum 67	Proximal 19 Distal 53	Caecum/ascending 38 Transversum 8 Descending colon/sigmoid 64 Rectum 95	NA	cecum and ascending 53 transverse 21 descending 18 sigmoid 110
Scoring Method	Intensity	percentage and intensity tissue section	intensity	intensity	intensity & extent	Quantitative	NA	percentage and intensity	percentage and intensity	percentage and intensity tissue section	percentages and intensity	NA	NA	NA	percentages and intensity	NA	NA	NA
Score range and location	Score 0-3 Nuclear (4-point scale)	Nuclear 0-1	Nuclear 0-3 (0-1)	Nuclear 0-1	Nuclear 0-1	Nuclear 0-1	Nuclear 0-1	Nuclear 0-5 & 0-3	Nuclear 0-2	Nuclear 0-1	NA	NA	Nuclear 0-3	NA	NA	NA	NA	NA

DOP	NA	NA	>10%	NA	>10 %	> 5%	5% cut off value	NA	NA	>50%	NA	≥30%	>10%	>70%	NA	>50%	>50%	NA
Antibody for IHC dilution	NA	1;100	NA	1;100	1;200	NA	1;200	1;800	1;100	1;50	1;500	1;100	1;100	1;200	1;200	NA	1;2000	1;100
Antibody Source	Santa Cruz	Santa Cruz	Invitrogen	Cell Signaling Technology	Santa Cruz	Dako	Dako	Biosciences 610154	Abcam	cell signaling technology	Dako	BD Bioscience,	Dako	Dako	BD Transduction Laboratories	BD Transduction Laboratories	Trasduction Laboratories	Transduction Laboratories
MV variables	NA	Age, Geneder, Location, Grade, TNM stage	Age, Size, Histologic grade, Stage (3/4 vs. 1/2), Lymphatic invasion, Perineural invasion, Venous invasion	Age, Tumour location, Tumor depth, Lymph node metastasis, Tumor differentiation, Lymphatic invasion,Venous invasion, Adjuvant chemotherapy, Wnt1, Wnt3, Wnt5a, Wnt8a, Membrane and Cytoplasm B-catenin expression.	sex, age, tumor diameter, location, differentiation, depth of invasion, lymph node metastasis, distant metastasis, and expression of KAI1/ CD82, CD44, MMP7.	Gender, location, differentiation, TNM stage, family history, MSI status, Age.	NA	Age,gender , stage, differentiation, location, resection, MSIstatus, E-cadherin expression, SOX9 expression.	NA	Gender, Age, Tumour Size, Type of Tumour, Differentiation, Invasion depth, Lymph node metastasis.	Age, location, TNM stage, Histologic grade, SIRT1.	NA	Gender, Primary location.	NA	PPARγ (Positive vs negative), Distant metastases	NA	NA	Age, Gender, Tumour stage.
HR (95% CI)	UV analysis 0.93 (0.45-1.93)	MV analysis 3.86(1.24 -11.99)	UV analysis 0.666 (0.449–0.986) MV analysis 0.740 (0.497–1.101)	UV analysis2.1 (1.2-3.8) MV analysis 1.9 (1.0-3.4)	MV analysis 1.652 (1.058-2.579)	UV analysis 2.586 (1.194 - 5.597) MV analysis 3.842 (1.422 - 10.376)	UV analysis 1.02(0.60-1.74)	UV analysis 1.11 (0.83–1.48) MV analysis 0.97 (0.71–1.34)	UV analysis	MV analysis (1.449–5.645)	UV analysis 0.493 (0.293-0.831)/ MV analysis 0.685 (0.392-1.198)	UV analysis 0.558 (0.339–0.918)	MV analysis 2.48 (1.30–4.74)	UV analysis	MV analysis 4.057 (1.507-10.918)	UV analysis	UV analysis	UV analysis 3.41 (1.62–7.17) MV analysis 2.34 (1.11–4.97)
P value	0.842	0.02	0.042 0.138	0.01 0.04	0.027	0.016 0.008	0.936	0.43 0.99	0.05	0.001	0.008 0.18	0.022	0.006	< 0.05	0.006	0.0339	0.028	0.0012 0.0264

HR estimation	Survival table	Survival curve Survival table	Survival table Survival curve	Survival table	Survival table	Survival table Survival curve	Survival table	Survival table	Survival curve	Survival curve Survival table	Survival table	Survival curve Survival table	Survival table	Survival table	Survival table	Survival curve	Survival curve & reported in text	Survival table Survival curve

DOP; Definition of Positive, **NA**; Non applicable, **MV**; Multivarate , **UV**; Univariante, **N**; Nuclear, **HR**; Hazard Ratio, **95% CI**;95% confidence Interval

Table 4. Studies characteristics and the impact of membrane β -catenin expression on Overall Survival in Colorectal Cancer.

Author	Tóth [12]	Andras [32]	Zhang [34]	Boo [33]
Year	2012	2012	2008	2007
Country	Hungary	Hungary	Japan	Korea
Patients (N)	79	91	77	138
Age (years)	Mean 65.8	Mean 66.9	Mean 64.9	Mean 57.9
Gender (M/F)	40/39	52/48	51/26	79/59
Follow up (months)	Mean 52	Mean 50.9	NA	Median 70.9
Neo adjuvant /Adjuvant Therapy	adjuvant	adjuvant	NA	adjuvant
Cancer related death n(%)	NA	NA	NA	NA
Beta-catenin expression n	27 M	55 M	42 M	47 M
Tumour stage			NA	Stage I-II-III&IV
Tumour site	Right sided colon 19 Left sided colon 29 Rectum 31	Right sided colon 25 Left sided colon 38 Rectum 37	NA	Colon 71 Rectum 67
Scoring Method	NA	percentages and intensity	NA	NA
Score range and location	NA	NA	NA	NA
DOP	>10%	>10%	>70%	<80%
Antibody for IHC dilution	1;100	1;100	1;200	
Antibody Source	Transduction Laboratories,	Transduction Laboratories	BD Transduction Laboratories	Dako
HR (95% CI)	UV analysis	UV analysis	UV analysis	UV analysis
P value	0.28	0.012	0.044	0.028
HR estimation	Reported in the text Survival curve	Survival curve	Survival curve Survival table	Survival curve Survival table

DOP Definition of Positive, **NA**, Non applicable, **UV** Univariante. **N** Nuclear, **HR** Hazard Ratio, **95% CI** 95% confidence Interval, there is no row for MV variable because all the studies have done UV analysis only.